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## Hepatotoxicity following administration of onasemnogene abeparvovec (AVXS-101) for the treatment of spinal muscular atrophy

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**Abstract:** Background aims: Spinal muscular atrophy (SMA) is an autosomal recessive, childhood-onset motor neuron disease. Onasemnogene abeparvovec (OA) is a gene therapy designed to address SMA's root cause. In pivotal mouse toxicology studies, the liver was identified as a major site of OA toxicity. Clinical data reflect elevations in serum aminotransferase concentrations, with some reports of serious acute liver injury. Prophylactic prednisolone mitigates these effects. Herein, we aim to provide pragmatic, supportive guidance for identification, management, and risk mitigation of potential drug-induced liver injury. Methods: Data from 325 patients with SMA who had received OA through 31 December 2019, in 5 clinical trials, a managed access program (MAP), and a long-term registry (RESTORE), and through commercial use, were analyzed. Liver-related adverse events, laboratory data, concomitant medications, and prednisolone use were analyzed. Results: Based on adverse events and laboratory data, 90 of 100 patients had elevated liver function test results (alanine aminotransferase, and/or aspartate aminotransferase, and/or bilirubin concentrations). Of these, liver-associated adverse events were reported for 34 of 100 (34%) and 10 of 43 (23%) patients in clinical trials and MAP/RESTORE, respectively. Two patients in MAP had serious acute liver injury, which resolved completely. While all events in the overall population resolved, prednisolone treatment duration varied (range: 33-229 days), with a majority receiving prednisolone for 60-120 days. More than 60% had elevations in either alanine aminotransferase, aspartate aminotransferase, or bilirubin concentrations prior to dosing. Greater than 40% received potentially hepatotoxic concomitant medications. Conclusions: Hepatotoxicity is a known risk associated with OA use. Practitioners should identify contributing factors and mitigate risk through appropriate monitoring and intervention. Lay summary: Onasemnogene abeparvovec is a type of medicine called a "gene therapy," which is used to treat babies and young children who have a rare, serious inherited condition called "spinal muscular atrophy" (SMA). It works by supplying a fully functioning copy of the survival motor neuron or SMN gene, which then helps the body produce enough SMN protein. However, it can cause an immune response that could lead to an increase in enzymes produced by the liver. This article provides information about the liver injury and how to prevent and recognize if it happens, so that it may be treated properly.

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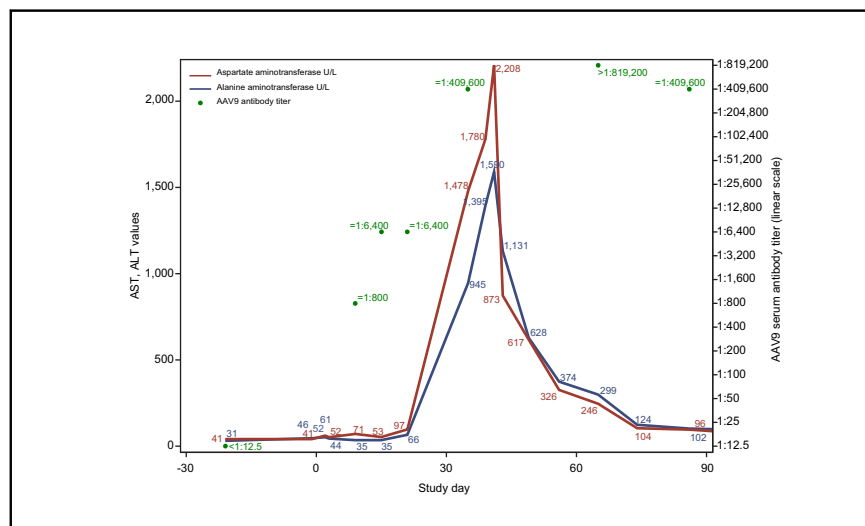
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# Hepatotoxicity following administration of onasemnogene abeparvovec (AVXS-101) for the treatment of spinal muscular atrophy

## Graphical abstract



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## Lay summary

Onasemnogene abeparvovec is a type of medicine called a “gene therapy,” which is used to treat babies and young children who have a rare, serious inherited condition called “spinal muscular atrophy” (SMA). It works by supplying a fully functioning copy of the survival motor neuron or SMN gene, which then helps the body produce enough SMN protein. However, it can cause an immune response that could lead to an increase in enzymes produced by the liver. This article provides information about the liver injury and how to prevent and recognize if it happens, so that it may be treated properly.

## Highlights

- Onasemnogene abeparvovec is an approved gene therapy for spinal muscular atrophy.
- Hepatotoxicity is a known risk associated with onasemnogene abeparvovec.
- Liver-related data for 325 patients who received onasemnogene abeparvovec were analyzed and are presented.
- The risk of hepatotoxicity can be serious if not recognized.
- Practitioners should mitigate risk through appropriate monitoring and intervention.

# Hepatotoxicity following administration of onasemnogene abeparvovec (AVXS-101) for the treatment of spinal muscular atrophy

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**Background & Aims:** Spinal muscular atrophy (SMA) is an autosomal recessive, childhood-onset motor neuron disease. Onasemnogene abeparvovec (OA) is a gene therapy designed to address SMA's root cause. In pivotal mouse toxicology studies, the liver was identified as a major site of OA toxicity. Clinical data reflect elevations in serum aminotransferase concentrations, with some reports of serious acute liver injury. Prophylactic prednisolone mitigates these effects. Herein, we aim to provide pragmatic, supportive guidance for identification, management, and risk mitigation of potential drug-induced liver injury.

**Methods:** Data from 325 patients with SMA who had received OA through 31 December 2019, in 5 clinical trials, a managed access program (MAP), and a long-term registry (RESTORE), and through commercial use, were analyzed. Liver-related adverse events, laboratory data, concomitant medications, and prednisolone use were analyzed.

**Results:** Based on adverse events and laboratory data, 90 of 100 patients had elevated liver function test results (alanine aminotransferase, and/or aspartate aminotransferase, and/or bilirubin concentrations). Of these, liver-associated adverse events were reported for 34 of 100 (34%) and 10 of 43 (23%) patients in clinical trials and MAP/RESTORE, respectively. Two patients in MAP had serious acute liver injury, which resolved completely. While all events in the overall population resolved, prednisolone treatment duration varied (range: 33–229 days), with a majority receiving prednisolone for 60–120 days. More than 60% had elevations in either alanine aminotransferase, aspartate aminotransferase, or bilirubin concentrations prior to dosing. Greater than 40% received potentially hepatotoxic concomitant medications.

**Conclusions:** Hepatotoxicity is a known risk associated with OA use. Practitioners should identify contributing factors and mitigate risk through appropriate monitoring and intervention.

**Lay summary:** Onasemnogene abeparvovec is a type of medicine called a “gene therapy,” which is used to treat babies and young children who have a rare, serious inherited condition called “spinal muscular atrophy” (SMA). It works by supplying a fully functioning copy of the survival motor neuron or *SMN* gene, which then helps the body produce enough SMN protein. However, it can cause an immune response that could lead to an increase in enzymes produced by the liver. This article provides information about the liver injury and how to prevent and recognize if it happens, so that it may be treated properly.

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## Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive, early childhood disease with an incidence of approximately 1:10,000 live births.<sup>1–3</sup> The genetic diagnosis of SMA is based on the presence of a survival motor neuron 1 or *SMN1* mutation or deletion. Phenotype was historically classified into 4 groups based on age at onset and greatest motor function achieved, with an additional phenotype (type 0) to describe the severe forms of antenatal onset.<sup>4</sup> Approximately 45–60% of cases have the most common phenotype, SMA type 1. Phenotype severity is largely determined by *SMN2* copy numbers, with a minor contribution from other genetic or environmental factors. Individuals born with 0 to 3 copies of *SMN2* in the presence of a bi-allelic *SMN1* deletion or mutation have a high probability of developing a severe phenotype.<sup>4</sup> Prior to the availability of effective treatment, SMA was the leading genetic cause of infant mortality.<sup>1,5</sup>

SMA is a motor neuron disease whose root cause is SMN protein deficiency.<sup>6</sup> Deficiency of this protein correlates directly with motor neuron death, resulting in the progressive loss of muscle control, strength and function, thereby leading to difficulty swallowing and breathing and, consequently, to death.<sup>7</sup> Although the primary pathology of SMA is neuronal, clinical reports indicate involvement of other organs, including the liver, heart, pancreas, and intestine.<sup>8</sup> SMA has been associated with impaired hepatic function, with more than one-third of

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autopsies of children with SMA revealing liver steatosis on post-mortem necropsy.

Zolgensma<sup>®</sup> (onasemnogene abeparvovec) has been approved for the treatment of SMA in the United States, Europe, Japan, Brazil, Israel, Canada, and Taiwan. Onasemnogene abeparvovec is a single-dose, intravenous gene therapy designed to address the monogenic root cause of SMA by utilizing a non-replicating, non-integrating, recombinant adeno-associated virus serotype 9 (AAV9) capsid to deliver a stable, fully functional human *SMN* transgene to increase *SMN* protein expression and prevent motor neuron cell death, leading to improved neuronal and muscular function. The *SMN* present in onasemnogene abeparvovec resides as a DNA episome in the nucleus of transduced cells, and appears to be highly stable in post-mitotic cells, such as motor neurons.

Clinical trials have demonstrated clear evidence of clinically meaningful efficacy following administration of onasemnogene abeparvovec in this otherwise devastating, neurodegenerative disease. Transient elevations in serum aminotransferase concentrations have been reported following the use of onasemnogene abeparvovec, although the exact mechanism of injury has not been elucidated. Preclinical studies have suggested an immune response to the vector capsid as a possible mechanism. Cell-mediated immunity directed against the AAV capsid plays an important role in safety and efficacy of AAV gene transfer in humans. Immune responses have been observed in AAV vector clinical trials across different neuromuscular diseases (e.g., SMA, Duchenne muscular dystrophy, myotubular myopathy). Transduction and subsequent expression of the AAV vector in fixed tissue macrophages in the liver, known as Kupffer cells, can elicit an immune response and effectively eliminate the therapeutic effect by developing antibodies to the capsid and/or transgene or eliminating the transduced cells through a cytotoxic T-cell-mediated mechanism.<sup>9</sup> Generation of capsid-specific T-cell responses may depend on the AAV serotype used, and the specificity of the AAV capsid variants may impact immune recognition.<sup>10</sup> Once a certain threshold of capsid antigen load has been reached, activation of capsid-specific T cells may result in hepatotoxicity in some cases.<sup>11,12</sup> Anti-capsid cytotoxic T-cell-mediated destruction of transduced hepatocytes in the clinical setting may be mitigated with prophylactic or on-demand immunosuppression. No immunosuppressive regimens were used in preclinical studies with onasemnogene abeparvovec.

Herein, we describe these liver effects, paying particular attention to clinical characteristics, with the aim of providing pragmatic, supportive guidance for the identification, management, and risk mitigation of potential drug-induced liver injury (DILI).

## Materials and methods

### Data sources

The data on liver abnormalities were from 2 basic sources: (1) 5 sponsored clinical trials of open-label treatment (summarized in the [supplementary information](#)) and (2) compassionate use and post-marketing data monitored by the sponsor. The data collected in the clinical trials were prospective and specified via protocol. Data from the compassionate use and post-marketing studies were *ad hoc* (i.e., reported voluntarily to the company). In the latter situation, attempts were made to obtain additional details from the reporting sources.

### Clinical trials

All studies were performed in accordance with ethical principles from the Declaration of Helsinki and are consistent with International Council for Harmonisation/Good Clinical Practice and applicable regulatory requirements. All study protocols were approved by the institutional review boards and appropriate informed consent was obtained.

As of 31 December 2019, 101 patients in 5 clinical studies (2 completed and 3 ongoing) had received a single intravenous dose of onasemnogene abeparvovec ([Table S1](#)). Studies were registered with [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (START: NCT02122952, STRIVE-US: NCT03306277, SPRINT: NCT03505099, STRIVE-EU: NCT03461289, and STRIVE-AP: NCT03837184). Study data were not available within this data cut-off for STRIVE-AP, and, therefore, the number of patients dosed for this analysis was 100.

Patients were eligible to participate if they had been genetically diagnosed with SMA type 1 and were <8 months of age (<180 days; or ≤6 weeks for the pre-symptomatic SPRINT) at the time of infusion.

Patients were excluded from clinical study participation if they had elevated (either >3 or >2× the upper limit of normal [ULN], depending on the protocol) baseline gamma-glutamyltransferase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), or total bilirubin values; anti-AAV9 antibody titer >1:50; prior or concomitant SMA-treating medications (e.g., nusinersen); or known allergy or hypersensitivity to glucocorticoids.

Eligible patients received a 1-time intravenous dose of onasemnogene abeparvovec. With the exception of the first 3 patients who received a lower dose in the Phase I START study, all other patients received the therapeutic dose of  $1.1 \times 10^{14}$  vg/kg in the START, STRIVE-US, SPRINT, and STRIVE-EU studies. For analytic purposes, all patients dosed were evaluated. Prophylactic administration of prednisolone was outlined in study protocols. Patients received approximately 1 mg/kg/day of prednisolone (or equivalent) 24 hours prior to dosing through at least 30 days post-administration. A recommended tapering schedule was provided in protocols, including guidelines for adjustment based on elevations in serum aminotransferase concentrations. In March 2019, in response to a report of acute liver failure, this prednisolone dosing schedule was revised. The revised regimen included initiation at 2 mg/kg/day, starting the day prior to dosing, for 3 days; and then reduction to 1 mg/kg/day for 30 days, with tapering. Modification to dosing and duration of glucocorticoids was left to the investigators' discretion.

Per study protocols, safety was assessed through reported adverse events, vital signs, cardiac and laboratory evaluations (chemistry, hematology, urinalysis, and immunology), physical examinations, and concomitant medications. Specifically, hepatotoxicity was evaluated using a combination of adverse events coded in accordance with Medical Dictionary of Regulatory Activities (MedDRA<sup>®</sup>; Version 21.0), and scheduled interval laboratory values. With standard nomenclature, adverse events were classified as serious if they met applicable criteria.<sup>13</sup> Serum aminotransferase concentrations were also evaluated using Hy's Law criteria.<sup>14</sup> Per protocol, serum antibody titers to AAV9 were collected and analyzed. Specific, pre-determined terms were used to systematically search for investigator-reported liver-related adverse events indicative of potential hepatotoxicity. To

comprehensively evaluate hepatotoxicity, we also evaluated laboratory data to assess abnormal values not reported as adverse events.

Study inclusion criteria excluded patients with mild baseline elevations. For the purpose of this current analysis, the number (%) of patients meeting the following predefined potential hepatotoxicity criteria was summarized as ALT and/or AST: i)  $\geq 3 \times$  ULN to  $< 5 \times$  ULN (mild); ii)  $\geq 5$  to  $< 20 \times$  ULN (moderate); and iii)  $\geq 20 \times$  ULN (severe).

### Open-access programs

Under special circumstances (*i.e.*, compassionate use), treatment with onasemnogene abeparvovec was initiated by physician request in the United States for patients with SMA type 1 older than 6 months of age. This initially occurred in the setting of an individual investigational new drug (IND) application held by individual physicians, and subsequently through a managed access program (MAP) protocol. The MAP terminated upon approval of onasemnogene abeparvovec by the US FDA on 24 May 2019.

The Prospective, Long-Term Registry of Patients with a Diagnosis of Spinal Muscular Atrophy (RESTORE) was initiated on 24 May 2018. It is an ongoing, global, observational registry designed to assess long-term outcomes, safety, and effectiveness of onasemnogene abeparvovec. Some patients in this registry received SMA treatment other than onasemnogene abeparvovec and are excluded from this analysis.

### Post-marketing data

After approval of onasemnogene abeparvovec in respective regions, safety data continues to be monitored through various avenues including spontaneous reports, literature, solicited venues, *etc.* Safety was assessed through reported adverse events. Each reported adverse event within the Hepatobiliary Disorders system organ class was reviewed.

### Statistical methods

Summary statistics for continuous data, including ALT, AST, and prednisolone, were analyzed as mean, standard deviation, median, minimum, and maximum. Plots were generated and statistical analyses were performed using SAS® 9.4 statistical software to assess the magnitude and duration of elevations in serum aminotransferase concentrations. These were plotted with respect to interval AAV9 antibody titers. Patient visit windows were applied per study protocols. All data were descriptive; no *a priori* hypothesis tests are provided.

## Results

### Clinical studies

Overall, 101 patients were enrolled into intravenous studies, of whom 100 were included in this analysis. Only 1 patient was enrolled in STRIVE-AP at the time of data cut-off; this patient was excluded from this analysis as a full clinical analysis of STRIVE-AP was unavailable. Of note, this patient continues in the study and has not had reported liver-associated adverse events or elevated serum aminotransferase concentrations. Demographics and clinical characteristics of the 100 patients analyzed are summarized in Table S2. Mean age for therapeutic dosing was 2.9 months, and 59% of patients were female. SPR1NT enrolled only pre-symptomatic patients, thereby representing a younger patient population (mean age = 0.8 months).

### Medical history and concomitant medications

At least 1 comorbid condition was reported for 53 of 100 patients (53%). The most common comorbidities were gastroesophageal reflux disease (12%), hypotonia (12%), atrial septal defect (9%), and eczema (5%). All other conditions were reported in  $< 5\%$  of patients.

Concomitant medications were defined as any medication started on or after the date of onasemnogene abeparvovec administration and were updated at each visit. Overall, 96 of 100 clinical study patients (96%) reported the use of at least 1 concomitant medication. The most commonly used concomitant medications were acetaminophen ( $> 40\%$ ), ibuprofen ( $> 20\%$ ), and ranitidine ( $> 20\%$ ).

Concomitant medication data were not collected in RESTORE or MAP, as they represent clinical practice. Similarly, post-marketing data were primarily from spontaneous self-reporting, and information on concomitant medication was often missing. Therefore, results cannot be provided for these sources.

### Prednisolone exposure

Ninety-nine of 100 patients received prophylactic prednisolone following a protocol revision after increased serum transaminase concentrations occurred in the first patient dosed in START, the initial phase I study.

As prednisolone treatment duration was at the discretion of the investigators, mean duration of prednisolone treatment was 83 days (range: 33–229) in all the studies, with a majority of patients receiving prednisolone for 60–120 days (Fig. 1, Table S3). As a result of this variability in dosage and duration, no correlation between prednisolone dosage and magnitude and duration of increases/decreases in serum aminotransferase concentrations were assessable.

### Adverse event overview

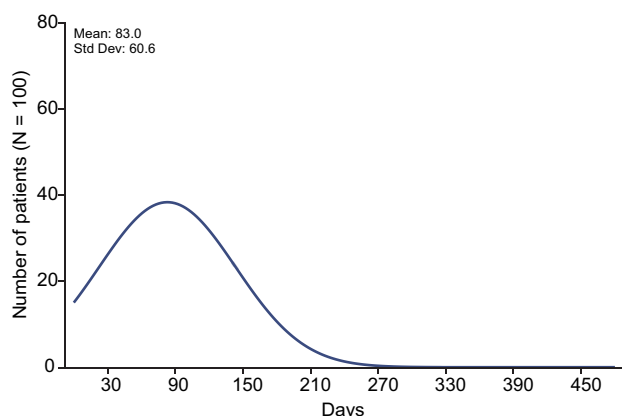
Of the 100 patients who received intravenous onasemnogene abeparvovec, 99 experienced at least 1 adverse event after dosing. Of these, 57 (57%) were considered by the investigator to have been related to onasemnogene abeparvovec. Moreover, 48 patients experienced serious adverse events, of which 11 were considered by the investigator to have been related to onasemnogene abeparvovec. The most frequently reported treatment-related adverse events and serious adverse events across all studies were elevations in serum aminotransferase enzyme concentrations and vomiting.

### Liver-specific evaluation

Of patients who received therapeutic intravenous dosing, 34 (34%) had at least 1 adverse event within the hepatotoxicity category. All adverse events associated with increased serum aminotransferase concentrations resolved completely, some with alterations in prednisolone dosing.

In analyzing laboratory data, we included any elevation after dosing, irrespective of baseline value. Of 100 children treated in clinical trials, 90% had at least some degree of ALT and/or AST elevation during therapy. While the majority of elevations were  $< 3 \times$  ULN, 9% were categorized as mild ( $\geq 3 \times$  ULN to  $< 5 \times$  ULN), 6% as moderate ( $\geq 5$  to  $< 20 \times$  ULN), and 5% as severe ( $\geq 20 \times$  ULN) (Table S4). No patient had elevations in ALT and/or AST concentrations, with concurrent elevations in bilirubin  $\geq 2 \times$  ULN in clinical trials. The elevations typically began at Week 1 after the





**Fig. 1. Distribution of prednisolone duration in onasemnogene abeparvovec clinical studies.**

infusion with a second peak at Month 1, at the time of the tapering and discontinuation of systemic corticosteroid therapy. Individual patient data with respect to onset and resolution days, outcomes, and respective prednisolone dosing in those with moderate to severe elevations are depicted in [Table S5](#).

Of note, 61 patients (61%) had elevations in ALT and/or AST and/or bilirubin concentrations prior to dosing. In the SPRINT trial, 23 of 30 (76.7%) pre-symptomatic patients had elevations in ALT and/or AST and/or bilirubin concentrations at baseline prior to dosing that were less than study exclusion thresholds, all of which normalized over time and remained normal at final study visit.

As of 31 December 2019, no clinical trial cases meeting clinical or biochemical criteria for Hy's law were observed.

The relationship between serum aminotransferases concentration elevations and anti-AAV9 antibody titers was explored. Elevations in serum aminotransferase concentrations, if present, were observed following antibody titer increases. As expected in all cases, antibody concentrations increased after onasemnogene abeparvovec administration and remained elevated and stable through data cut-off, irrespective of serum aminotransferase normalization. A schematic of the typical rise and fall in anti-AAV antibodies and serum aminotransferase concentrations for an individual patient representing a majority of the study patients is provided in [Fig. 2](#).

### Open-access programs

As of 31 December 2019, 54 patients were enrolled in US MAP and/or RESTORE combined: 11 of whom did not receive onasemnogene abeparvovec and were excluded from this analysis.

Of the 43 patients dosed with onasemnogene abeparvovec, 28 patients (65.1%) experienced adverse events, and 13 patients (30.2%) experienced serious adverse events. The most frequently reported treatment-related adverse events included ALT and/or AST concentration increases for 10 patients (23.3%); liver function test increases for 5 patients (11.6%; 4 of these overlap with ALT and/or AST increases); thrombocytopenia for 4 patients (9.3%); and vomiting for 2 patients (4.7%).

In many cases, neither concomitant use of prednisolone nor dosage were reported. However, the transaminase elevations resolved completely in all cases. Only 2 children (4.7%) developed concurrent bilirubin elevations, arising with severe

elevations in ALT and AST ( $>40\times$  ULN). Presenting bilirubin concentrations were 8.4 and 3.5 mg/dl and INR values were 6.6 and 1.5, respectively. Both children responded to re-initiation of prednisone therapy or dosage increase and ultimately could be withdrawn from immunosuppressive therapy without recurrence of hepatitis. Details on the 2 cases of liver injury reported in the MAP are presented in the [supplementary information](#).

### Post-marketing safety cases

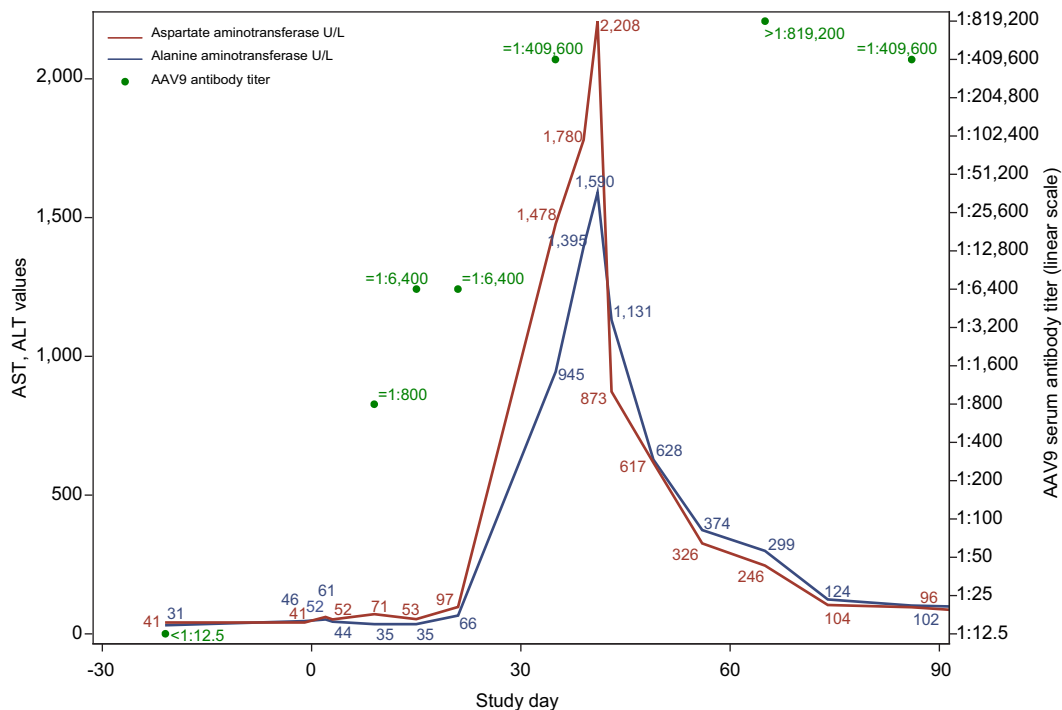
As data from the compassionate use and post-marketing studies were not collected in a structured manner, but rather provided unilaterally by the reporter, specific details (e.g., peak laboratory values, time to resolution, concomitant medications, etc.) were inconsistent and limited. As of 31 December 2019, 181 patients had received onasemnogene abeparvovec commercially, and 192 reports of 488 adverse events were retrieved from the Novartis Global Patient Safety database. Individual patients often had multiple reports and events at various time points. Most events contained limited information regarding concomitant medications, outcome, medical history, etc. The most frequently reported events included pyrexia (50), vomiting (42), hepatic enzyme increased (24), AST increased (23), ALT increased (19), liver function test increased (17), platelet decreased (16), troponin increased (7), thrombocytopenia (7), cough (7), nasopharyngitis (7), and weight decreased (6).

### Discussion

The term SMA is applied to a diverse group of genetic disorders, of which the most common form results from bi-allelic mutations in the *SMN1* gene on chromosome 5q13.<sup>2,15</sup> A deletion or mutation of both copies of *SMN1* results in decreased expression of the SMN protein.<sup>16</sup> The *SMN2* copy number is inversely related to the phenotypic continuum of clinical severity. A greater number of copies is associated with less severe disease.<sup>17,18</sup>

Children with SMA have been reported to have abnormal fatty acid metabolism, which was reported to cause liver failure in a patient with SMA type 2, who required orthotopic liver transplant.<sup>19</sup> The authors attributed the sensitivities to abnormal  $\beta$ -oxidation (e.g., dicarboxylic aciduria) which appeared to be unique to SMA compared with non-SMA denervating disorders, and correlated with clinical severity, as infants with severe SMA have significant abnormalities in concentrations of fatty acid metabolites. Similarly, Brehm *et al.*<sup>20</sup> reported on a patient with SMA and liver injury after receiving therapeutic doses of acetaminophen. They postulated that patients with decreased muscle mass were susceptible to acetaminophen toxicity because of decreased stores of glutathione, as well as reduced volume of distribution, thus leading to increased plasma acetaminophen concentrations.<sup>20</sup> In addition, more than one-third of autopsies of children with SMA exhibited evidence of liver steatosis.<sup>20</sup> While non-alcoholic steatohepatitis is a diagnostic consideration for adult patients with acute liver injury, non-alcoholic steatohepatitis is extremely rare in very young children.<sup>21</sup>

In pivotal toxicology studies of neonatal FVB/NJ mice, the liver was identified as one of the main sites of onasemnogene abeparvovec toxicity. Liver findings were noted at doses above the clinical recommended intravenous dose of  $1.1 \times 10^{14}$  vg/kg onasemnogene abeparvovec. Increases in serum aminotransferase (ALT, AST) concentrations were noted at 3, 6, and/or 12 weeks post intravenous injection, with evidence of reversibility by Week 12. These transient elevations in serum aminotransferase



**Fig. 2. AAV antibody titer concentrations compared with liver function test values for average patient representative of population.** Y-axis retains same scale through entire study by patient. The axis is not reset for each graphing interval. Y2-axis is on a linear scale (not Log Base 2 scale). Each dilution on the Y2-axis is double the preceding dilution. Patients' titer results are included within the plot. A <1:50 titer is considered as negative. AAV9, adeno-associated virus serotype 9; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

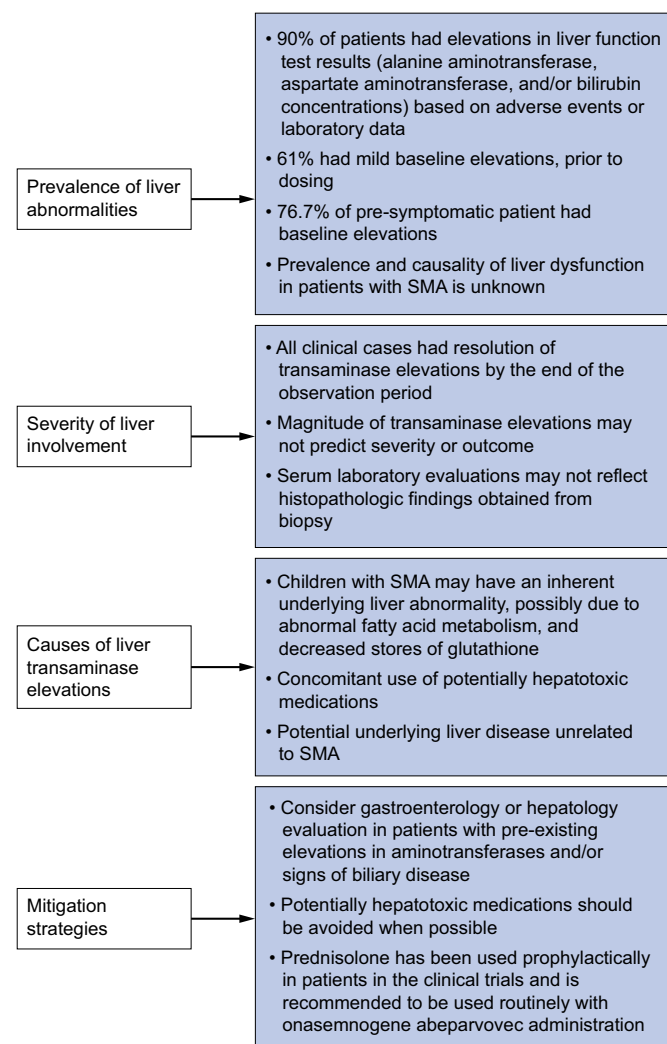
concentrations were suggestive of hepatocellular damage and correlated with histopathology findings composed of hepatocellular hypertrophy, Kupffer cell activation, and scattered hepatocellular necrosis. In a recently published study, a single intravenous injection of  $2.0 \times 10^{14}$  vg/kg of an AAV9-like vector (AAVhu68) carrying the SMN transgene to juvenile rhesus macaques, resulted in significant systemic inflammation and liver toxicity including transaminase elevations.<sup>22</sup> The mechanism of onasemnogene abeparvovec-related liver damage is unknown, but given the liver is among the most transduced tissues, these liver effects are likely associated with hepatocellular uptake of AAV.

Two patients (see [supplementary information](#)) also described by Feldman AG *et al.*,<sup>23</sup> met the diagnostic criteria for DILI, but not solely for drug-induced liver failure because of confounding factors (e.g., pre-existing elevations and family history in Case 1, and lack of jaundice, encephalopathy, or impairment of liver synthetic function in Case 2). Both patients were treated with methylprednisolone and were discharged from the hospital within 2 weeks of initial diagnosis. Both patients underwent biopsy and exhibited inflammatory infiltrates composed of CD8<sup>+</sup> T cells (Case 1 in the periportal areas and Case 2 as mild interface hepatitis). Case 1 had fibrosis on biopsy; repeat liver biopsy performed 2 months later revealed resolution of inflammation but persistence of fibrosis. Serum aminotransferase concentrations had normalized at the time of repeat biopsy, raising the question of pre-existing liver disease.

Pre-existing liver enzyme elevations, especially in SMA children, may represent underlying liver disease, a predisposition to acute liver injury.<sup>19</sup> Clinical norms should be based on age-appropriate limits, rather than laboratory defined reference

ranges. Based on the combination of adverse event and laboratory data, 90 of 100 patients had some degree of elevations in ALT and/or AST and/or bilirubin concentrations, and 61 (61%) had elevations in ALT and/or AST and/or bilirubin concentrations, prior to dosing. Of the pre-symptomatic study patients, 23 (76.7%) had some degree of elevations in ALT and/or AST and/or bilirubin concentrations at baseline, which raises the possibility of underlying liver abnormalities. Despite these baseline elevations, the prevalence and causality of inherent liver dysfunction in patients with SMA are unknown. Serum AST and bilirubin concentrations must be interpreted carefully during infancy as physiologic changes can be misinterpreted as liver injury.<sup>24</sup> Although the primary feature of SMA is neurodegeneration, some clinical reports indicate that other organs are involved, including the liver, heart, pancreas, and intestine, some of which may contribute to enzymatic release.<sup>8</sup> Red blood cells contain AST<sup>25</sup> and the increased turnover because of physiologic transition from fetal to adult hemoglobin may cause AST concentrations to be up to 6-fold greater for infants than for adults.<sup>26,27</sup> Should patients have elevations in aminotransferase concentrations and/or signs of biliary disease prior to dosing, practitioners should consider potential etiologies based on clinical presentation, signs and symptoms, age, and medical and family histories. Darras *et al.*<sup>28</sup> and Feldman *et al.*<sup>23</sup> have described serum aminotransferase concentration elevations with nusinersen administration and the combination of onasemnogene abeparvovec with nusinersen administration within 1 month, respectively. Nusinersen is an antisense oligonucleotide, which increases production of full-length SMN protein by modifying splicing in available SMN2 pre-mRNA, and is given via repeat intrathecal dosing to treat the symptoms of SMA.<sup>29</sup> Potentially





**Fig. 3. Hepatotoxicity with onasemnogene abeparvovec use in patients with SMA.** SMA, spinal muscular atrophy.

hepatotoxic medications should be avoided if possible, before administration of onasemnogene abeparvovec and within 1 month after dosing, as liver enzyme elevations have been mainly described within 1 month after dosing with onasemnogene abeparvovec.

In the 5 clinical studies, the transient increases in mean ALT and AST values occurred at approximately Day 7, with a return to near baseline concentrations at approximately Day 14. Second transient increases in mean ALT and AST values were observed at Month 1, with return to near baseline concentrations by Month 2 ([supplementary information](#)). All serum aminotransferase concentration elevations normalized by the end of the study observation periods. While commercial use of the product confirmed this temporal relationship, actual laboratory values with respect to time to resolution were often not provided in this setting.

Given the mechanism of hepatotoxicity is presumed to be immune-mediated, prednisolone was used empirically in the first patient dosed to treat aminotransferase transaminase concentrations to complete resolution. Since then, it has been used

prophylactically in all clinical trial patients and is recommended to be used with onasemnogene abeparvovec administration as standard of care. In the clinical trials, investigators were allowed to use discretion with prednisolone dosing and duration based on their clinical judgments for each individual patient. Liver histopathology was not obtained in the clinical trials and pre-clinical studies have not been conducted to evaluate the effects of prednisolone in this setting. Hence, no conclusive recommendations can be made on optimal prednisolone dosage or duration in response to aminotransferase concentration elevations.

In summary, hepatotoxicity related to onasemnogene abeparvovec use typically presents as non-cholestatic (*i.e.*, as elevations in serum aminotransferases concentrations). Some children have presented with elevations as high >20× ULN in ALT and/or AST, most often occurring at 1 week and 1 month after onasemnogene abeparvovec dosing. This should be anticipated. Accordingly, any potential contributors including underlying disease state and hepatotoxic concomitant medications should be appropriately investigated and avoided if possible. Prednisolone should be used prophylactically in accordance with prescribing guidelines and greater dosages and/or longer duration considered in the setting of post-dose elevations in serum aminotransferase concentrations. Consultation with a pediatric gastroenterologist/hepatologist should be considered as clinically appropriate. A summary of hepatotoxicity associated with onasemnogene abeparvovec use in SMA patients is presented in [Fig. 3](#).

### Abbreviations

AAV9, adeno-associated virus serotype 9; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DILI, drug-induced liver injury; IND, investigational new drug; MAP, managed access program; SMA, spinal muscular atrophy; SMN, survival motor neuron; ULN, upper limit of normal; US FDA, United States Food and Drug Administration.

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### Conflicts of interest

DC, KM, AK, RS, FM, ST-W, FFT, GK-U and PK are employees of Novartis, and own Novartis stock or other equities. HJM has participated in ad boards and medical symposia for Novartis Gene Therapies (formerly AveXis, Inc.).

Please refer to the accompanying [ICMJE disclosure](#) forms for further details.

### Authors' contributions

Concept and design: DC, FM, ST-W, PK, FFT. Data collection: DC, ST-W, PK. Data analysis: DC, AK, KM, RS, FFT. Drafting of the manuscript: DC, FM, HM, GK-U. Statistical analysis: AK. Critical

revision of the manuscript: DC, FM, HM, PK, ST-W, FFT, KM, AK, GK-U, RS.

### Data availability statement

Novartis is committed to sharing clinical trial data with external researchers and has been doing so voluntarily since 2014. Novartis was the third member to join [ClinicalStudyDataRequest.com](https://www.clinicalstudydatarequest.com) (CSDR), which is the first data sharing consortium of clinical study sponsors and funders. CSDR is a leader in the data sharing community inspired to drive scientific innovation and improve medical care by facilitating access to patient-level data from clinical studies. More information is available at <https://www.novartisclinicaltrials.com/TrialConnectWeb/voluntarydataviewmore.nov>.

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### Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2020.11.001>.

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